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(71) Applicant *(for all designated States except US)*: CENTRAL BLOOD LABORATORIES AUTHORITY [GB/GB]; The Crest, Dagger Lane, Elstree, Borehamwood, Hertfordshire WD6 3AU (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : HUGHES-JONES, Nevin, Campbell [GB/GB]; 65 Orchard Road, Melbourn, Royston, Hertfordshire SG8 6BB (GB).

(74) Agents: HOLMES, Michael, John et al.; Frank B. Dehn & Co., Imperial House, 15-19 Kingsway, London WC2B 6UZ (GB).

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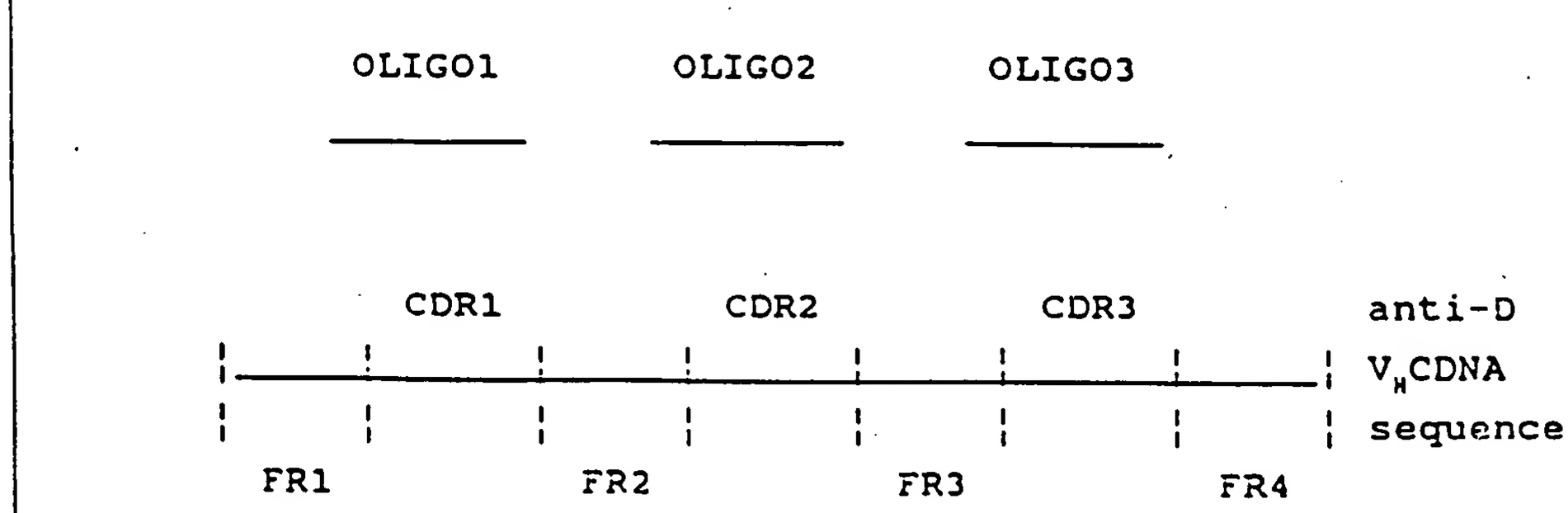


(57) Abstract

The present invention provides DNA sequences encoding complementarity determining regions of variable domains of human anti-RhD antibodies and their use in the production of recombinant chimaeric antibody molecules.

Monoclonal Antibodies

This invention relates to novel monoclonal anti-RhD antibodies prepared by recombinant DNA methods.

The Rhesus blood group system is a major antigenic constituent of the human red blood cell membrane; of this group, the RhD antigen is of particular clinical importance in relation to isoimmune reactions. An Rh D-individual with anti-RhD who receives RhD+ blood is liable to suffer substantial red blood cell (RBC) destruction due to the RhD phenotype incompatibility, and thus blood of donors must routinely be classified as RhD+ or RhD-. Anti RhD monoclonal antibodies (antiD Mabs) are capable of providing blood-typing reagents of high specificity and reliability.

The RhD antigen is also responsible for haemolytic disease of the newborn (HDN). This condition arises in newborn RhD+ infants of RhD- mothers previously sensitised to RhD antigen as a result of IgG anti-RhD antibodies crossing the placenta during pregnancy and causing foetal red blood cell (RBC) destruction. Sensitization of the RhD- mother to RhD antigen often occurs during the birth of an earlier RhD+ child due to some foetal RBCs entering the maternal circulation and being recognised as foreign by the maternal immune system. To reduce the incidence of HDN, it is routine practice in the United Kingdom and many other countries to give anti-RhD antibodies to RhD- mothers immediately after the birth of an RhD+ infant so that any RhD+ RBCs which may have entered the maternal circulation are rapidly removed.

The search for the most effective anti D Mabs has proved to be extremely time consuming, involving the isolation of B-lymphocytes from humans immunised against RhD, usually Rh-ve mothers who have given birth to Rh+ve

children. Such lymphocytes are subjected to EBV treatment to provide an immortalised cell-line directly or the EBV-treated cells are hybridised with suitable mouse myeloma cells to provide a hydridoma: The cell-line or hybridoma may then be used to produce the anti-D Mab in the conventional way.

However, there are significant differences between anti-D Mabs in terms of their binding affinities for red cells, their ability to recognise D-variants such as D^{VI} and D^{VI}, and their ability to destroy target cells by phagocytosis or cell-mediated lysis. It is desirable, therefore, to have available a method of combining the favourable parameters of different anti-D Mabs or, indeed of combining the most favourable features of selected anti-D Mabs with Mabs of quite different specificities which present particular advantages, in order to produce so-called chimaeric Mabs.

The concept of building chimaeric Mabs, has been described by Jones et al (Nature 321, 522-525 (1986)) and Riechmann et al (Nature 332, 323-327 (1988)). Three dimensional studies have shown that immunoglobulins comprise essentially constant regions common to most Mabs and terminally situated variable domains associated with antigen binding.

It has been shown that the variable domains consist of two β-sheets joined by a disulphide bridge with their hydrophobic faces in contact. Sequence comparisons among heavy- and light-chain variable domains (V_H and V_L respectively) have revealed that each of these domains comprises three hypervariable domains or complementarity determining regions (CDRs) set in a framework of four relatively conserved regions, the framework regions (FRs). The CDRs are primarily responsible for the recognition of specific antigens. The structure of the β-sheet framework is similar in different antibodies, as the packing together of V_L and V_H FRs is conserved and therefore the orientation of V_L with respect to V_H is

fixed.

Genes coding for a number of Mabs are now available and the sequences coding for the variable regions V_L and V_H have been determined. It is thus possible to replace the latter sequences by DNA coding for V_L and V_H from different Mabs and indeed to construct the latter by incorporating DNA coding for chosen CDRs into DNA coding for a standard set of FRs. It is thus possible to construct genes coding for chimeric anti-D Mabs having the CDRs from anti-D Mabs possessing particularly desirable specificities or other properties and framework and constant regions derived from Mabs having other desirable properties.

It is a prerequisite of such construction that the amino acid sequences of the CDR regions of the chosen anti-D Mabs and/or the genes coding for them, should be known. The specific CDR gene sequences can then be synthesised, conveniently by chemical synthesis of the appropriate oligonucleotides, and incorporated into DNA sequences coding for a standard set of FRs and the human (or other) constant region. Of course, the FRs may be identical with those of the Mab providing the constant region or, more conveniently, they may be a standard set of FRs which can be used generally in the synthesis of chimeric Mabs.

We have produced a number of anti-D Mabs of particular interest and have determined their amino acid sequences, thus making it possible for DNA sequences corresponding to their CDRs to be synthesised and incorporated into V_H and V_L sequences as described above. These may then be combined with DNA coding for the constant region to enable novel anti-D Mabs to be synthesised which may have lower, the same or higher binding ability.

Thus, according to one aspect we provide DNA sequences comprising oligonucleotides encoding CDR1, CDR2, and CDR3 regions of V_H and V_L domains of antibodies

against the human RhD antigen, and functional equivalents thereof. In particular, we have investigated and sequenced eleven Mabs, namely a) FOG-B, b) PAG-1, c) MAD-2, d) FOG-1, e) FOM-1, f) FOM-A, g) BRAD-3, h) JAC-10, i) GAD-2, J) REG-A, K) HAM-B, whose heavy and light chain sequences are represented in figures 2-14, of the accompanying drawings, and which have both varied and particularly useful binding specificities. The figures 2 and 3 show the nucleotide and amino acid sequences of the light chain variable domains of the Mabs FOG-B and PAG-1. Corresponding sequences for the heavy chain variable domains of these two Mabs are shown in figures 4 and 5, and sequences of the heavy chain variable domains of the Mabs MAD-2, FOG-1, FOM-A, BRAD-3, JAC-10, GAD-2, REG-A and HAM-B are shown in figures 6-14.

Synthetic genes, for both heavy and light chains may be created by combining selected CDR 1, 2, and 3 regions, which may be selected from different antibody molecules having varied binding specificities.

Thus according to a further aspect, we provide DNA molecules coding for the heavy or light chain fragments of a monoclonal antibody or fragment thereof comprising CDR1, CDR2 and CDR3 encoding oligonucleotides from antibodies FOG-B, PAG-1, MAD-2, FOG-1, FOM-1, FOM-A, BRAD-3, JAC-10, GAD-2, REG-A and HAM-B as illustrated in figures 2-14.

In order to create functional genes, such oligonucleotides must be incorporated into a backbone sequence such that when expressed, functional proteins result.

Thus according to a further aspect, we provide DNA molecules comprising a gene coding for the framework regions of a human antibody light or heavy chain having inserted therein in the correct CDR region, oligonucleotides encoding CDR1, CDR2 and CDR3 regions according to the present invention.

In the synthesis of a chimeric Mab in accordance with the invention, single stranded DNA coding for the V_H region of a chosen Mab (not necessarily an anti-D Mab) is incorporated in single stranded form into a vector capable of producing single stranded DNA, such as the M13 bacteriophage. Fig. 1 shows diagrammatically the structure of a single stranded V_H DNA including framework regions FR1 to FR4 with complementarity determining regions CDR1 to CDR3 of a Mab. These steps can be accomplished by conventional techniques such as those described in Riechmann et al (Nature, 332, 323-327, (1988)).

Three oligonucleotides may then be prepared corresponding to the CDR regions of the chosen anti-D Mab variable domain, eg the V_H region of FOG-B as shown in Fig. 4, and will include several nucleotides on either side of each CDR region to permit hybridisation with the framework regions FR1 to FR4 (see figure 1). The sequences of the latter will normally be substantially homologous with those of the anti-D Mab (e.g. FOG-B) but since the oligonucleotides will normally be synthesised chemically, hybridisation may be ensured by matching the overlapping nucleotides exactly to the FRs 1 to 4. It may also be beneficial to modify the oligonucleotides to express the CDRs more efficiently in the eventual host cells.

The three oligonucleotides, shown in Fig 1 as oligo 1 to oligo 3, may then be annealed to a single stranded V_H DNA in the M13 vector and used as primers to synthesise second strand DNA containing the anti-D V_H CDR sequences. This may be achieved conventionally using a suitable polymerase. Since the antibody specificity is determined solely by the three CDR regions, the actual V_H gene chosen for the framework template is immaterial. All that is required is that there is sufficient homology of the three chosen oligonucleotides with the template. This is ensured by appropriate design of the

terminal nucleotides of the synthetic oligonucleotide primers. Thus the second strand may contain sequences from substantially any human antibody heavy chain gene, so long as the resulting expressed protein posesses the desired binding parameters.

The double stranded M13 vector may then be used to transform a suitable host microorganism e.g. a conventional E. coli and one or more clones selected which contain the required anti-D V_H specificity. The correct clone may be identified by DNA seqencing.

The corresponding V_L DNA (e.g. for FOG-B) may be prepared in the same way.

The DNA coding for the V_H and V_L regions may then be excised from the above vectors and introduced into other vectors.

According to a further aspect, we provide DNA molecules being synthetic genes for chimaeric antibody, heavy or light chains when incorporated into vectors capable of expressing such antibody chains. Preferred vectors include mammalian expression vectors, such as pSV2gpt (heavy chains) and pSV2neo (light chains) containing DNA coding for the human constant region. Such vectors are readily available from a number of laboratories, or can readily be prepared by incorporating DNA coding for human constant region into known mammalian vectors.

The expression vectors so constructed may then be co-transfected into an appropriate cell-line e.g. a non-secreting IgG myeloma, for large scale production.

Thus according to a yet further aspect, the present invention provides each of the CDR polypeptides of the Mabs FOG-B, PAG-1, MAD-2, FOG-1, FOM-1, FOM-A, BRAD-3, JAC-I0, GAD-2, REG-A and HAM-B shown in Figs. 2-14 of the accompanying drawings in single stranded or double stranded form in the absence of the constant and or framework regions of said Mabs.

According to a yet further aspect, the invention

provides chimaeric antibody heavy and light chains of the variable domains comprising CDR polypeptide sequences of the present invention.

Knowledge of the antibody sequences according to the invention enables new chimaeric anti-D antibody molecules to be prepared, having appropriately designed binding specificities. These antibodies may be used for both therapy and diagnosis using presently known techniques.

According to a yet further aspect, we provide anti-RhD reagents comprising at least one antibody molecule according to the invention.

According to a still yet further aspect, we provide pharmaceutical compositions for use in passive immunisation to prevent haemolytic disease of the newborn comprising an antibody of the present invention together with at least one phamacologically acceptable carrier or diluent.

A sterile solution of such an antibody for human injection may be formulated in any physiologically acceptable aqueous medium, for example isotonic phosphate buffered saline or serum. Alternatively, the antibody may be supplied in a freeze-dried formulation ready for reconstitution prior to use.

EXAMPLE

(1) Construction of Chimaeric Antibody Genes

Three oligonucleotide primers are synthesised using an Applied Biosystems machine according to the manufacturer's instructions and purified on an 8 M Urea/polyacrylamide gel (Sanger & Coulson, Febs Lett., 87, 107-110, 1978). The primers are designed to comprise in their central regions sequences complementary to the CDR1, CDR2 and CDR3 regions of the anti-RhD antibody PAG-1 heavy chain gene, as identified according to the criteria described by Kabat et al. (Sequences of Proteins of Immunological Interest, US Department of Health and Social Services, 1987).

The central sequences are flanked at both their 5' and 3' termini by sequences of 10 nucleotides which hybridise to the termini of the corresponding framework region sequences adjacent to the CDR sequence of the heavy chain antibody gene NEWM (Poljack et al., Biochemistry 16, 3412-3420, 1977). The primers are then hybridised to the derived NEWM single stranded DNA heavy chain sequence in the M13 bacteriophage and the complementary strand of the heavy chain variable region extended using DNA polymerase (Neuberger et al., Nature 314, 268-270 (1985), Jones et al., Nature 321, 522-5 (1986)). The M13 vector also contains an appropriate arrangement for ultimate expression, i.e. a leader sequence, and unique HindIII and BamHI restriction sites.

A similar construct is prepared from oligonucleotide primers homologous to the CDR regions of the PAG-1 anti-RhD antibody light chain genes, and utilising the M13 vector in which V_L and J_L regions of the antibody gene PAV1 (Sun et al., Nucleic Acids Research 13, 4921-4934, 1985) are cloned.

(2) Expression of Antibody Polypeptides

The cloned genes for the V_H domains are excised using HindIII and BamHI and cloned into pSV2gpt (Mulligan and Berg, PNAS 78, 2072-6, 1981). The cloned light chain genes are similarly excised and cloned into pSV2neo (Southern and Berg, J. Molec. Appl. Genetics 1 327-381, 1981). Sequences encloding IgG1 constant regions are then inserted into the vectors (Riechmann et al., Nature 312, 323-7, (1988). Both vectors are then transfected by electroporation (Potter et al., PNAS 81, 7161-3, 1984) into the rat myeloma cell line YO (YB2/3.0 AG, 20) (Galfre and Milstein, Methods in Enzymology 73, 1-46, 1981) for antibody production.

CLAIMS

1. A DNA sequence comprising an oligonucleotide encoding a CDR1, CDR2 and/or CDR3 region of a V_H or V_L domain of an antibody against the human RhD antigen, and functional equivalents thereof.

2. A DNA sequence as claimed in claim 1 encoding the CDR1 region of a V_H domain selected from:

AGTGGTGGTCTCTACTGGGGC;
AGTTCCTACTGGAGC;
GGTTACTACTGGAGC;
GTTTACTACTGGACC;
GGTTACTACTGGAAC;
GGTTACTACTGGAGC;
AGCTATGGCATGCAC;
AGTTACTGGATGCAC;
AGCTATGGCATGCAC;
AATTATGGCATGCAC; and
AGCTATGGCATGCAC,

optionally with extended terminal regions.

3. A DNA sequence as claimed in claim 1 encoding the CDR2 region of a V_H domain selected from:

AGTATATTTTATAGTGGGAGCACCTACTACAATCCCTCCCTCAAGA3C;
TATATCTATTACAGTGGGAGCACCAACTACAACCCCTCCCTCAGGAGT;
GAAATCAATCATAGTGGAAGGACCAACTACAACCCGTCCCTCAAGACT;
GAAATCAATCATAGTGGAGGCGCCAACTACAATCCGTCCCTCAAGAGT;
GAAATCATTCATAGTGGAAGCACCAACTACAACCCGTCCCTCAAGAGT;
GAAATCAGTCGTCGTGGAAGCACCAACTACAACCCGTCCCTCAAGAGT;
CTTATATGGTATGATGGAAGTAATAAAGAATATGCAGACTTCGTGAAG GGC;
CGTATTAATAGTTATGGAATTAGCACAAGTTACGCGAACTCCGTGAAG GGC;

GTGATATGGTATGATGGAAGTAATAAGTACTATGCAGAGTCCGTGAAG GGC;

GTTATATGGTATGATGGAAGTAATAAAAACTATGCAGACTCCGTGAAG GGC; and

GTTATTTGGTATGATGGAAGTAATAAATACTATGCAGACTCCGTGAAG GGC,

optionally with extended terminal regions.

4. A DNA sequence as claimed in claim 1 encoding the CDR3 region of a V_H domain selected from:

CCAGGCTATGGCGACACCTCGGTACGGAAGAGGGTTTGGAATATGGAC CTC;

GTTTTGGTTTCCCGTACCATTTCACAGTACTCCTATTACATGGACGTC;

GTTTTGGTTTCCCGTACGATTTCACAGTACTCCTATTACATGGACGTC;

CTGTGGCTCGATGGACATGGGTACAAGTTTGACTAC;

GGCCGGTCCCGTTATAGTGGTTACGGCTTCTACTCCGGCATGGACGTC;

GGCTTAGAACGTCCGATTAGGAACCAGCTGCTAAACCGTCTCGGTTAC TACATGGACGTC;

GCCTTGGACTACATCTCCTTGGATTACGGTATGGACG C;

GATAGTCCCAAAATGAGGGCTGGAAGTATGTTTCGCT CTACTACATG GACGTC;

GGAGAGCGCATAGCAGCTCGTCTCTTGTCGGGCGGGT CGGTATGGAC GTC;

GTCGTTAGCAGCAACCGGTACTCTCTAAGCTACTATT TTACTACATGGAC GTC;

GAACGTACTACGATGTCTGGAGTGATCATTCCTCGCCG GTATTTTGAC TAC; and

GAAGTTACTATGGTTCGGGGAGTTAGGCGTTACTACGGTATGGACGTC,

optionally with extended terminal regions.

5. A DNA sequence as claimed in claim 1 encoding the CDR1 region of a V_L domain selected from:

TCCGGAACCAGCTCCAACATTGGGAATAATTATGTATCC;

GGGGGAAACAACATTGGGCGTAAAAGTGTGCAC; and
GGGGGAAACAACATTGGACGTAAAAGTGTGCAC,

optionally with extended terminal regions.

6. A DNA sequence as claimed in claim 1 encoding the CDR2 region of a V_L domain selected from:

GACAATAATAAGCGACCCTCA;
GGTGCTAGCGAGCGGCCCTCA; and
GGTGCTAGCGACCGGCCCTCA,

optionally with extended terminal regions.

7. A DNA sequence as claimed in claim 1 encoding the CDR3 region of a V_L domain selected from:

GCAACATGGGATAGCAGCCTGAGTGCTGTGGTG; and
CAGGTGTGGGATAGTAGTAGTGCTCATCCGGGGGTGGTA,

optionally with extended terminal regions.

8. A DNA sequence as claimed in any one of claims 2 to 7 wherein the said extended terminal regions hybridise with the terminal sequences of the framework regions of a human antibody heavy or light chain gene flanking the CDR region.

9. A DNA molecule for the synthesis of a synthetic gene coding for the heavy chain fragment of a monoclonal antibody or fragment thereof comprising a CDR1 encoding oligonucleotide as claimed in claim 2, a CDR2 encoding oligonucleotide as claim in claim 3 and a CDR3 oligonucleotide as claimed in claim 4.

10. A DNA molecule for the synthesis of a synthetic gene coding for the light chain fragment of a monoclonal antibody or fragment thereof comprising a CDR1 encoding oligonucleotide as claimed in claim 5, a CDR2 encoding oligonucleotide as claimed in claim 6 and a CDR3 oligonucleotide as claimed in claim 7.

11. A DNA molecule comprising a gene coding for the framework region of a human antibody light or heavy chain having inserted therein for a heavy chain in the CDR1 position an oligonucleotide as claimed in claim 2, in the CDR2 position, an oligonucleotide as claimed in claim 3 and in the CDR3 position, an oligonucleotide as claimed in claim 4.

12. A DNA molecule comprising a gene coding for the framework region of a human antibody light or heavy chain having inserted therein for a light chain in the CDR1 position an oligonucleotide as claimed in claim 5, in the CDR2 position an oligonucleotide as claimed in claim 6 and in the CDR3 position an oligonucleotide as claimed in claim 7.

13. A DNA molecule as claimed in claim 11 or claim 12 when incorporated in a vector capable of expressing the said antibody heavy or light chain.

14. An expression vector as claimed in claim 13 which is replicable in mammalian cells.

15. A polypeptide sequence encoded by a CDR nucleotide sequence as claimed in any one of claims 2 to 7, and functional equivalents thereof.

16. A chimaeric antibody V_H or V_L chain or fragment thereof encoded by a DNA sequence as claimed

respectively in claim 11 or claim 12.

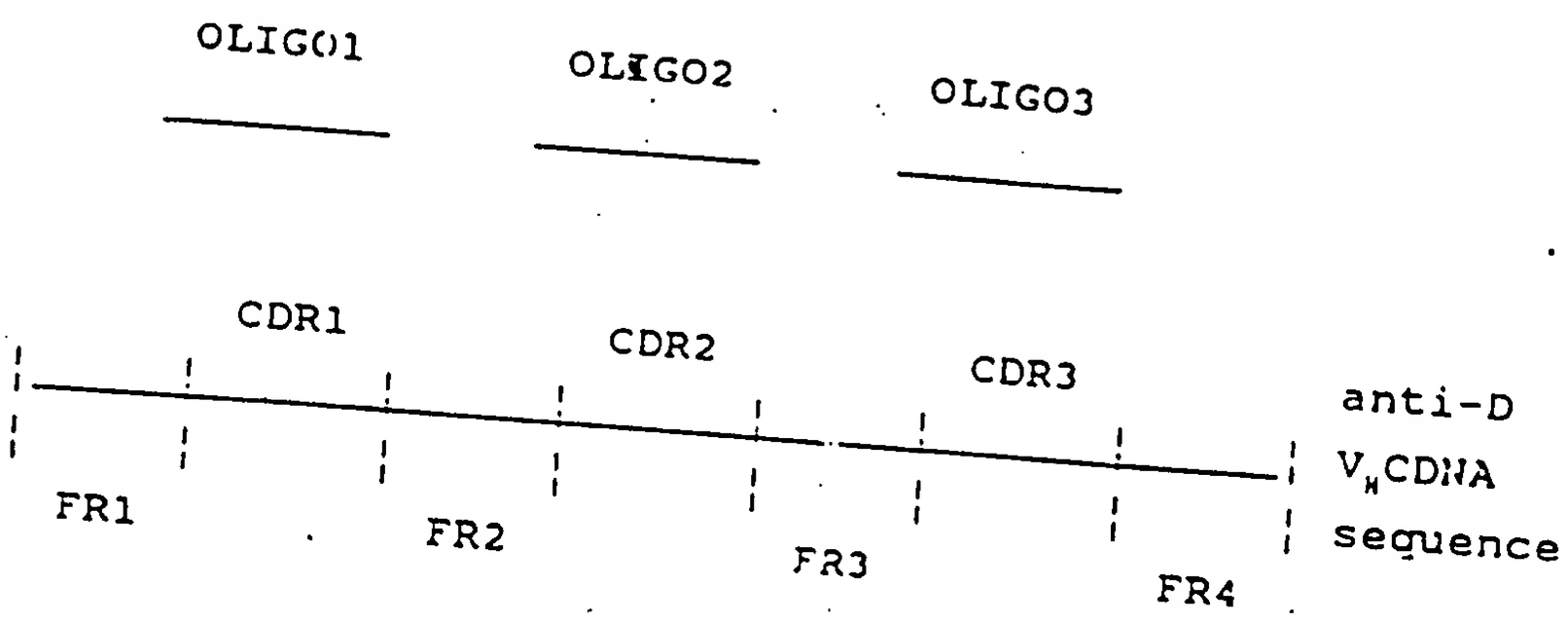
17. A chimaeric antibody molecule against the RhD antigen wherein the variable regions of the heavy and light chains comprise polypeptide sequences as claimed in claim 15.

18. An anti-RhD reagent comprising at least one antibody molecule as claimed in claim 17.

19. A pharmaceutical composition for use in passive immunisation to prevent haemolytic disease of the newborn comprising an antibody as claimed in claim 17 together with at least one pharmacologically acceptable carrier or diluent.

20. A method of Rh-typing wherein an antibody as claimed in claim 17 is employed.

FIG. 1


OLIGO1
OLIGO2
OLIGO3
CDR1
CDR2
CDR3
FR1
FR2
FR3
FR4
anti-D
V_HCDNA
sequence

FOG-B VL SEQUENCE

SUBSTITUTE SHEET

```
  1  CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTCACCATC   60
     Q  S  V  L  T  Q  P  P  S  V  S  A  A  P  G  Q  K  V  T  I

 61  TCCTGCTCTGGGAACCAGCTCCAGCATTGGGAATAATTATGTATCCTGGTATCAGCAGCTC  120
     S  C  S  G  T  S  S  N  I  G  N  N  Y  V  S  W  Y  Q  Q  L
           <----------------------CDR1---------------------->

121  CCAGGAACAGCCCCCAAACTCCTCATTTATGACAATAATAAGCGACCCTCAGGGATTCCT  180
     P  G  T  A  P  K  L  L  I  Y  D  N  N  K  R  P  S  G  I  P
                                 <----------CDR2---------->

181  GACCGATTCTCTGGCTCCAAGTCTGGCACGTCAGCCACCCTGGGCATCACCGGACTCCAG  240
     D  R  F  S  G  S  K  S  G  T  S  A  T  L  G  I  T  G  L  R

241  ACTGGGGACGAGGCCGATTATTACTGCGGAACATGGGATAGCAGCCTGAGTGCTGTGGTG  300
     T  G  D  E  A  D  Y  Y  C  G  T  W  D  S  S  L  S  A  V  V
                                 <----------CDR3---------->

301  TTCGGCGGAGGGACCAAGCTGACCGTCCTAAGT  333
     F  G  G  G  T  K  L  T  V  L  S
```

VL SEQUENCE


```
1   TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAGGATT   60
    S  Y  V  L  T  Q  P  P  S  V  S  V  A  P  G  Q  T  A  R  I

61  ACCTGTGGGGGAAACAACATTGGACGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGC  120
    T  C  G  G  N  N  I  G  R  K  S  V  H  W  Y  Q  Q  K  P  G
                <----------CDR1----------->

121 CAGGCCCCTGTGCTGGTCGTCTATGGTGCTAGCGACCGGCCCTCAGGGATCCCTGAGCGA  180
    Q  A  P  V  L  V  V  Y  G  A  S  D  R  P  S  G  I  P  E  R
                      <----------CDR2----------->

181 TTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGCAGCCGGG  240
    F  S  G  S  N  S  G  N  T  A  T  L  T  I  S  R  V  A  A  G

241 GATCAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAGTGCTCATCCGGGGGTGGTA  300
    D  E  A  D  Y  Y  C  Q  V  W  D  S  S  S  A  H  P  G  V  V
                            <----------CDR3----------->

301 TTCGGCGGAGGGACCAAGCTGACCGTCCTAGGT  333
    F  G  G  G  T  K  L  T  V  L  G
```

FOG-B

4/14

VH SEQUENCE

```
1    CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTC    60
     Q  L  R  L  Q  E  S  G  P  G  L  V  K  P  S  E  T  L  S  L

61   ACCTGCAGTGTCTCTGGTGGCTCCGTCAGCAGTGGTGGTCTCTACTGGGGCTGGGTCCGC   120
     T  C  S  V  S  G  G  S  V  S  S  G  G  L  Y  W  G  W  V  R
                                  <--------CDR1--------->

121  CAGCCCCCAGGGAAGGGGCTCGAATGGATTGGCAGTATATTTTATAGTGGGAGCACCTAC   180
     Q  P  P  G  K  G  L  E  W  I  G  S  I  F  Y  S  G  S  T  Y
                                  <----------------CDR2-----

181  TACAATCCCTCCCTCAAGAGCCGAGTCACCATATCCGTAGACACGTTGAAGAATAACTTC   240
     Y  N  P  S  L  K  S  R  V  T  I  S  V  D  T  L  K  N  N  F
     ------------------------>

241  TCCCTGAAGCTGAGTTCTGTGACCGCCGCAGACACGGCTGTTTATTACTGTACGAGACCA   300
     S  L  K  L  S  S  V  T  A  A  D  T  A  V  Y  Y  C  T  R  P
                                                        <--

301  GGCTATGGCGACACCTCGGTACGGAAGACGGTTTGGACTATGGACCTCTGGGGCCAAGGG   360
     G  Y  G  D  T  S  V  R  K  R  V  W  T  M  D  L  W  G  Q  G
     ------------------CDR3------------------------------------>

361  ACCACGGTCACCGTCTCCTCG   381
     T  T  V  T  V  S  S
```

```
1    CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCGTC    60
     Q  V  Q  L  Q  E  S  G  P  G  L  V  K  P  S  E  T  L  S  V

61   ACCTGCACTGTCTCTGGTGGCTCCGTCAGTAGTTCCTACTGGAGCTGGATCCGGCAGCCC   120
     T  C  T  V  S  G  G  S  V  S  S  S  Y  W  S  W  I  R  Q  P
                                  <------CDR1------>

121  CCAGGGAAGGGACCGGAGTGGATTGGGTATATCTATTACAGTGGGAGCACCAACTACAAC   180
     P  G  K  G  P  E  W  I  G  Y  I  Y  Y  S  G  S  T  N  Y  N
                                  <-------------------------CDR2-----
                                                            *

181  CCCTCCCTCAGGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTG   240
     P  S  L  R  S  R  V  T  I  S  V  D  T  S  K  N  Q  F  S  L
     ---------------->
                  *

241  AAGCTGGGCTCTGTGACCGCTGCGGACACGGCCGTGTATTACTGTGCGAGAGTTTTGGTT   300
     K  L  G  S  V  T  A  A  D  T  A  V  Y  Y  C  A  R  V  L  V
                                                  <--------

301  TCCCGTACGATTTCACAGTACTCCTATTACATGGACGTCTGGGGCAAAGGGACCACGGTC   360
     S  R  T  I  S  Q  Y  S  Y  Y  M  D  V  W  G  K  G  T  T  V
     ----------CDR3------------------------------>

361  ACCGTGTCCTCA  372
     T  V  S  S
```

```
1    ------PCR primer--------CGCAGGACTGTTGAAGCCTTCGGAGACCCTGTCCCTC    60
          .  .  .  .  .  .  .  .  .  A  G  L  L  K  P  S  E  T  L  S  L

61   ACCTGCGCTGTCTATGGTGGGTCCTTCAGTGGTTACTACTGGAGCTGGATCCGCCAGCCT    120
     T  C  A  V  Y  G  G  S  F  S  G  Y  Y  W  S  W  I  R  Q  P
                                  <-----CDR1---->

121  CCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCAATCATAGTGGAAGGACCAACTACAAC    180
     P  G  K  G  L  E  W  I  G  E  I  N  H  S  G  R  T  N  Y  N
                                  <-------------------------CDR2-----

181  CCGTCCCTCAAGACTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTG    240
     P  S  L  K  T  R  V  T  I  S  V  D  T  S  K  N  Q  F  S  L
     ---------------->

241  AAGCTGAGTTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGACTGTGGCTC    300
     K  L  S  S  V  T  A  A  D  T  A  V  Y  Y  C  A  R  L  W  L
                                                  <--------

301  GATGGACATGGGTACAAGTTTGACTACTGGGGCCAGGGAACCCT---PCR primer---    360
     D  G  H  G  Y  K  F  D  Y  W  G  Q  G  T  L  .  .  .  .  .
     ------CDR3---------------->
```

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```
1    CAGGTGCATCTACAGCAGTGGGGCACAGGGCTGTTGAAGCCTTCGGAGACCCTGTCCCTC     60
     Q  V  H  L  Q  Q  W  G  T  G  L  L  K  P  S  E  T  L  S  L

61   ACCTGCGCTGTCCATGGTGGGTCCTTCAATGTTTACTACTGGACCTGGATCCGCCAGCCC    120
     T  C  A  V  H  G  G  S  F  N  V  Y  Y  W  T  W  I  R  Q  P
                                <-----CDR1----->

121  CCAGGAAAGGCGCTGGAGTGGATTGGGGAAATCAATCATAGTGGAGGCGCCAACTACAAT    180
     P  G  K  A  L  E  W  I  G  E  I  N  H  S  G  G  A  N  Y  N
                                <-------------------------CDR2-----

181  CCGTCCCTCAAGAGTCGAGTCACCATGTCAGCAGACACGTCCAAGAACCAGTTCTCCCTG    240
     P  S  L  K  S  R  V  T  M  S  A  D  T  S  K  N  Q  F  S  L
     ---------------->

241  AAACTGACCTCTGTGACCGCCGCGGACACGGCTGTGTTTTATTGTGCGAGAGGCCGGTCC    300
     K  L  T  S  V  T  A  A  D  T  A  V  F  Y  C  A  R  G  R  S
                                                <---------

301  CGTTATAGTGGTTACGGCTTCTACTCCGGCATGGACGTCTGGGGCCCAGGGACCACGGTC    360
     R  Y  S  G  Y  G  F  Y  S  G  M  D  V  W  G  P  G  T  T  V
     ----------------CDR3----------------------->

361  ACCGTCTCCTCA  372
     T  V  S  S
```

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VH SEQUENCE

SUBSTITUTE SHEET

```
1    CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTGTCCCTC     60
     Q  V  Q  L  Q  Q  W  G  A  G  L  L  K  P  S  E  T  L  S  L

61   ACCTGCGCTGTCTATGGTGGGTCCTTCAGTGGTTACTACTGGAACTGGATCCGCCAGCCC    120
     T  C  A  V  Y  G  G  S  F  S  G  Y  Y  W  N  W  I  R  Q  P
                                <------CDR1---->

121  CCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCATTCATAGTGGAAGCACCAACTACAAC    180
     P  G  K  G  L  E  W  I  G  E  I  I  H  S  G  S  T  N  Y  N
                                <-------------------------CDR2-----

181  CCGTCCCTCAAGAGTCGAGTCACCATGTCAGTAGACACGTCCAAGAACCAGTTCTCCCTG    240
     P  S  L  K  S  R  V  T  M  S  V  D  T  S  K  N  Q  F  S  L
     ------------------>

241  AAGCTGAGCTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGAGGCTTAGAA    300
     K  L  S  S  V  T  A  A  D  T  A  V  Y  Y  C  A  R  G  L  E
                                                   <--------

301  CGTCCGATTAGGAACCAGCTGCTAAACCGTCTCGGTTACTACATGGACGTCTGGGGCAAA    360
     R  P  I  R  N  Q  L  L  N  R  L  G  Y  Y  M  D  V  W  G  K
     ---------------CDR3----------------------------------------->

361  GGGACCACGGTCACCGTCTCCTCA  384
     G  T  T  V  T  V  S  S
```

FOM-A VH SEQUENCE

```
1    CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTGTCCCTC    60
     Q  V  Q  L  Q  Q  W  G  A  G  L  L  K  P  S  E  T  L  S  L

61   ACCTGCGCTGTCTATGGTGGGTCCTTCAGTGGTTACTACTGGAGCTGGATCCGCCAGCCC   120
     T  C  A  V  Y  G  G  S  F  S  G  Y  Y  W  S  W  I  R  Q  P
                                  <-----CDR1---->

121  CCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCAGTCGTCGTGGAAGCACCAACTACAAC   180
     P  G  K  G  L  E  W  I  G  E  I  S  R  R  G  S  T  N  Y  N
                                  <-----------------------CDR2-----

181  CCGTCCCTCAAGAGTCGAGTCGCCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTG   240
     P  S  L  K  S  R  V  A  I  S  V  D  T  S  K  N  Q  F  S  L
     ---------------->

241  AAGGTGAGGTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGAGCCTTGGAC   300
     K  V  R  S  V  T  A  A  D  T  A  V  Y  Y  C  A  R  A  L  D
                                                  /--------

301  TACATCTCCTTGGATTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCC   360
     Y  I  S  L  D  Y  G  M  D  V  W  G  Q  G  T  T  V  T  V  S
     ----------CDR3---------------->

361  TCA  363
     S
```

```
1    ------PCR primer-------GGGAGGCGTGGTCCAGCCTGGGAGGTTCCTGAGACTC   60
     . . . . . . . . . G G V V Q P G R F L R L

61   TCCTGTGCAGCGTCTGGATTCACCTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCT  120
     S C A A S G F T F S S Y G M H W V R Q A
                          <-----CDR1---->

121  CCAGGCAAGGGGCTGGAGTGGGTGGCACTTATATGGTATGATGGAAGTAATAAAGAATAT  180
     P G K G L E W V A L I W Y D G S N K E Y
                          <----------------------CDR2-----

181  GCAGACTTCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATACACTGTAT  240
     A D F V K G R F T I S R D N S K N T L Y
     ------------------>

241  CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGACAGATAGT  300
     L Q M N S L R A E D T A V Y Y C A T D S
                                          <-----

301  CCCAAAATGAGGGCTGGAAGTATGTTTCCC...TACCATGGACGTCTGGGGCAAAGGG  360
     P K M R A G S M F R Y Y Y M D V W G K G
     ------------------CDR3---------------------------->

361  ACCAC---PCR primer---  381
     T . . . . . .
```

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```
1    ------PCR primer--------GGGAGGCTTAGTTCAGCCTGGGGGGTCCCTGAGACTC   60
                             G  G  L  V  Q  P  G  G  S  L  R  L

61   TCCTGTGCAGCCTCTGGATTCACCTTCAGTAGTTACTGGATGCACTGGGTCCGCCAAGCT   120
     S  C  A  A  S  G  F  T  F  S  S  Y  W  M  H  W  V  R  Q  A
                                  <-----CDR1---->

121  CCAGGGAAGGGGCTGGTGTGGGTCTCACGTATTAATAGTTATGGAATTAGCACAAGTTAC   180
     P  G  K  G  L  V  W  V  S  R  I  N  S  Y  G  I  S  T  S  Y
                                  <-------------------------CDR2------

181  GCGAACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGCTGTAT   240
     A  N  S  V  K  G  R  F  T  I  S  R  D  N  A  K  N  T  L  Y
     ---------------->

241  CTGCAAATGAACACTCTGAGAGCCGAGGACACGGCTCTGTATTACTGTGCAAGAGGAGAG   300
     L  Q  M  N  N  T  L  R  A  E  D  T  A  V  Y  Y  C  A  R  G  E
                                                    <------

301  CGCATAGCAGCTCGTCTCTTGTCGGGCGGGTACGGTATGGACGTCTGGGGCCAAGGGACC   360
     R  I  A  A  R  L  L  S  G  G  Y  G  M  D  V  W  G  Q  G  T
     ------------------CDR3---------------------->

361  AC---PCR primer---  378
     .  .  .  .  .  .
```

GAD-2 VH SEQUENCE

```
1    ------PCR primer-------GGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTC   60
     . . . . . . . . . G G V V Q P G R S L R L

61   TCCTGTGCAGCGTCTGGATTCACCTTTAGTAGCTATGGCATGCACTGGGTCCGCCAGGCT   120
     S C A A S G F T F S S Y G M H W V R Q A
                          <-----CDR1---->

121  CCAGGCAAGGGGCTGGAGTGGGTGGCAGTGATATGGTATGATGGAAGTAATAAGTACTAT   180
     P G K G L E W V A V I W Y D G S N K Y Y
                          <----------------------CDR2-----

181  GCAGAGTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT   240
     A E S V K G R F T I S R D N S K N T L Y
     ----------------->

241  CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGTCGTT   300
     L Q M N S L R A E D T A V Y Y C A R V V
                                          <-----

301  AGCAGCAACCGGTACTCTCTAAGCTACTATTATTACTACATGGACGTCTGGGGCAAAGGG   360
     S S N R Y S L S Y Y Y Y Y H D V W G K G
     ----------CDR3----------------------->

361  ACCAC---PCR primer---  381
     T . . . . .
```

SUBSTITUTE SHEET

REG-A 13/14
VH SEQUENCE

```
1    ------PCR primer--------GGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTC    60
     . . . . . . . . . G G V V Q P G R S L R L

61   TCCTGTGCAGCGTCTGGATTCACCTTCAATAATTATGGCATGCACTGGGTCCGCCAGGCT    120
     S C A A S G F T F N N Y G M H W V R Q A
                              <-----CDR1---->

121  CCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTATGATGGAAGTAATAAAGACTAT    180
     P G K G L E W V A V I W Y D G S N K N Y
                              <----------------------CDR2-----

181  GCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT    240
     A D S V K G R F T I S R D N S K N T L Y
     ----------------->

241  CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGAACGT    300
     L Q M N S L R A E D T A V Y Y C A R E R
                                              <------

301  ACTACGATGTCTGGAGTGATCATTCCTCGCCGGTATTTTGACTACTGGGGCCAGGGAACC    360
     T T M S G V I I P R R Y F D Y W G Q G T
     ----------CDR3---------------------->

361  CG---PCR primer---  378
     . . . . . .
```

HAM-B VH SEQUENCE

```
1   ------PCR primer--------GGGRGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTC    60
    . . . . . . . . . G G V V Q P G R S L R L

61  TCCTGTGCAGCGTCTGGATTCACCTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCT    120
    S C A A S G F . F S S Y G M H W V R Q A
                      <-----CDR1---->

121 CCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATTTGGTATGATGGAAGTAATAAATACTAT    180
    P G K G L E W V A V I W Y D G S N K Y Y
                      <----------------------CDR2-----

181 GCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT    240
    A D S V K G R F T I S R D N S K N T L Y
    ---------------->

241 CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGAAGTT    300
    L Q M N S L R A E D T A V Y Y C A R E V
                                          <-----

301 ACTATGGTTCGGGGAGTTAGGCGTTACTACGGTATGGACGTCTGGGGCCCAGGGACCAC-    360
    T M V R G V R R Y Y G M D V W G P G T .
    ---------------CDR3---------------------->

361 --PCR primer---  375
    . . . . .
```

INTERNATICNAL SEARCH REPORT

International Application No PCT/EP 90/01964

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC C 12 N 15/13.

IPC[5]: C 07 K 15/28, C 12 P 21/08, A 61 K 39/395, G 01 N 33/80

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 12 N, C 12 P, C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	GB, A, 2189506 (CENTRAL BLOOD LABORATORIES AUTHORITY) 28 October 1987 see the whole document	15-20
Y	---	1-14
Y	EP, A, 0239400 (G.P. WINTER) 30 September 1987 see the whole document, especially page 31	1-14

A	Clinical Chemistry, volume 34, no. 9, September 1988, S.L. Morrison et al.: "Production and characterization of genetically engineered antibody molecules", pages 1668-1675 see the whole document	1-14
	--- ./.	

- Special categories of cited documents: [10]
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
5th February 1991	27 FEB 1991
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	LOUISE T. HALLAAR

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, " with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	Bio Essays, volume 8, no. 2, February/ March 1988, M. Verhoeyen et al.: "Engineering of antibodies", pages 74-78 see the whole document ---------	1-14